

Figure 1

Pfu	MILDVDYITTEGKPKVIRLFEKENGKPKIEHDIRPFIYALLRDDSKILEVKKITGERHG	10	20	30	40	50	60
DeepVent	MILDADYITTEGKPKIIRLFEKENGKPKIEHDIRPFIYALLRDDSKILEVKKITGERHG						
Hybrid_design	MILDVDYITTEGKPKVIRLFEKENGKPKIEHDIRPFIYALLRDDSKILEVKKITGERHG						
Pfu	KIVRIIVDVEKVEKKFLGKPTIVWKLYLEHPQDVPIREKVRHPAVVDIFPEYDIPPAKRY	70	80	90	100	110	120
DeepVent	KIVRIIDAEKVRKKFLGRPIEVWRRLYFEHPQDVPAIRDKIREHSAVIDIFPEYDIPPAKRY						
Hybrid_design	KIVRIIXDVEKVEKKFLGXPIIXVWXLXEHQDVPIREKVRHPAVVDIFPEYDIPPAKRY						
Pfu	LIDKGLIPMEGEELKILAFDIETLYHEGEELGKGPJIMISYADENEAKVITWKNIDLPY	130	140	150	160	170	180
DeepVent	LIDKGLIPMEGEELKILAFDIETLYHEGEELGKGPJIMISYADEEAKVITWKNIDLPY						
Hybrid_design	LIDKGLIPMEGXELKILAFDIETLYHEGEELGXKGPJIMISYADENEAKVITWKNIDLPY						
Pfu	VEVVSSEEMIKRFLRIIREKDDPIIVTYNGDSFDFFYLAKRAEKLOIKLIGRDGSEPK	190	200	210	220	230	240
DeepVent	VEVVSSEEMIKRFLKVIIEKDDPIIVTYNGDSFDFFYLAKRAEKLOIKLIGRDGSEPK						
Hybrid_design	VEVVSSEEMIKRFLXXIIEKDDPIIVTYNGDSFDFFYLAKRAEKLOIKLIGRDGSEPK						
Pfu	MQRIGDMTAVEVKGRIHFDLYHVIIRRTINLPTTYTLEAVYEAIFGKPKKVVADIEIAKAW	250	260	270	280	290	300
DeepVent	MQRIGDMTAVEIKGRIHFDLYHVIIRRTINLPTTYTLEAVYEAIFGKPKKVVADIEIAKAW						
Hybrid_design	MQRIGDMTAVEVKGRIHFDLYHVIIRRTINLPTTYTLEAVYEAIFGKPKKVVADIEIAKAW						
Pfu	SGENLERVAKYSMEDAKTYELGKERLPMELQLSRLVGGPLWDVSRSSSTGNLVWFLLRK	310	320	330	340	350	360
DeepVent	TGKGLERVAKYSMEDAKTYELGKERLPMELQLSRLVGGPLWDVSRSSSTGNLVWFLLRK						
Hybrid_design	XGXNLERVAKYSMEDAKTYELGXERLPMEXQLSRLVGGPLWDVSRSSSTGNLVWFLLRK						
Pfu	AYERNEVAPNKESELEYQRRRLRESYTGQFVKEPEKOLWENIWLDFRALYPSIITHNYS	370	380	390	400	410	420
DeepVent	AYERNELAPNKESELEYERRRLRESYAGQYVKEPEKOLWENIWLDFRALYPSIITHNYS						
Hybrid_design	AYERNEVAPNKESELEYQRRRLRESYTGQFVKEPEKOLWENIWLDFRALYPSIITHNYS						
Pfu	PDTLNLEGGCKNYDIAPQVGHKFKCDIPGFIPLLEGHLEERQKIKTKMKETQDPIEKILL	430	440	450	460	470	480
DeepVent	PDTLNLEGGCKNYDVAPQVGHKFKCDIPGFIPLLEGHLEERQKIKTKMKETQDPIEKILL						
Hybrid_design	PDTLNLEGGCKNYDXAPQVGHKFKCDIPGFIPLLEGHLEERQKIKTKMKETQDPIEKILL						
Pfu	DYRQKAIKLLANSFYGYGYAKARWYCKECAESVTAWGRNIEFLNWKLEEEKFGFKVLYI	490	500	510	520	530	540
DeepVent	DYRQKAIKLLANSFYGYGYAKARWYCKECAESVTAWGRNIEFLNWKLEEEKFGFKVLYI						
Hybrid_design	DYRQKAIKLLANSFYGYGYAKARWYCKECAESVTAWGRNIEFLNWKLEEEKFGFKVLYI						
Pfu	DTDGLYATIPGLESSEIKKKALEFVKYINSKLPOLLLELEYEGFYKGFVTKKXAYVIDE	550	560	570	580	590	600
DeepVent	DTDGLYATIPGAKPSEIKKKALEFVDYINAKLPOLLLELEYEGFYKGFVTKKXAYVIDE						
Hybrid_design	DTDGLYATIPGXXXSEIKKKALEFVKYINXKLPOLLLELEYEGFYKGFVTKKXAYVIDE						
Pfu	EGKVIITRGLEIVRRDWEIAKETQARNVLETILKHGDVEEAVRIYKEVIOKLIANVEIPPEK	610	620	630	640	650	660
DeepVent	EGKVIITRGLEIVRRDWEIAKETQAKVLEAILKHGNVEEAVKIYKEVTEKLSKYEIPPEK						
Hybrid_design	EGKVIITRGLEIVRRDWEIAKETQAXVLEXILKHGXVEEAVXIVREVVXXKLXXYEIPPEK						
Pfu	LAIYEQITRPLHEYKAIGPHVAVAKRLAAKGVKIKPGMVIGYIVLRGDPISNRALLAEE	670	680	690	700	710	720
DeepVent	LVIYEQITRPLHEYKAIGPHVAVAKRLAARGVKVVRPGMVIGYIVLRGDPISNRALLAEE						
Hybrid_design	LXIYEQITRPLHEYKAIGPHVAVAKXLAAXGVKXXPGMVIGYIVLRGDPISNRALLAEE						
Pfu	YDIPKXHKYDAEYYIENQVLPAYLRILEGFGYRKEDLRVOKTRQVGLTSLWNKKKS	730	740	750	760	770	780
DeepVent	FDLRKHKKYDAEYYIENQVLPAYLRILEAFGYRKEDLRVOKTRQVGLTSLWNKKKS						
Hybrid_design	XDXXKXHKYDAEYYIENQVLPAYLRILEXFGYRKEDLRVOKTXQXGLTSLWNKKKSGTHNC						
Pfu		790	800	810	820	830	840
DeepVent							
Hybrid_design	NHDI						

Figure 2. Assembly of the oligonucleotides into library fragments.

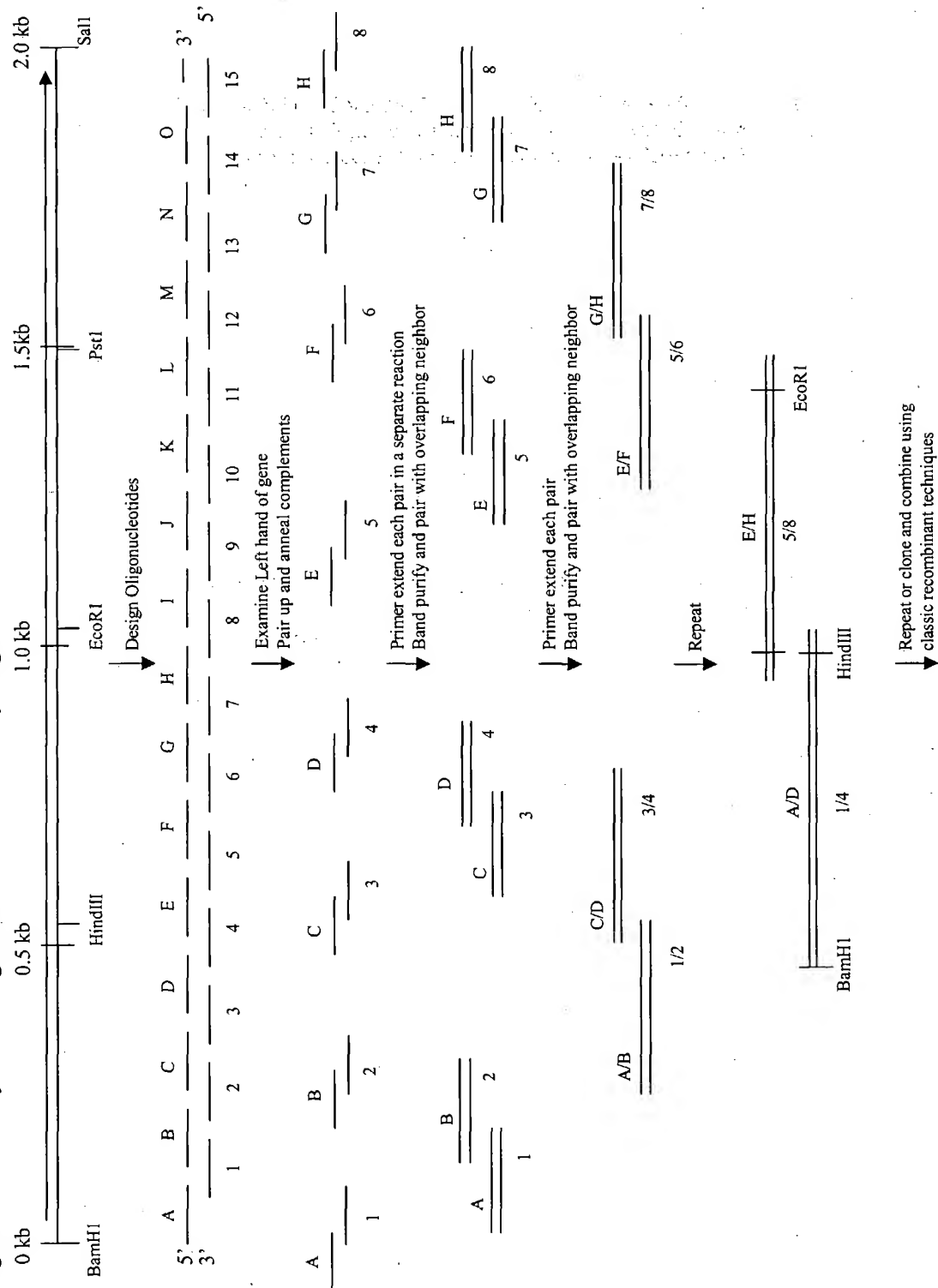


Figure 3. A comparison of the polymerase to 3' to 5' exonuclease activity

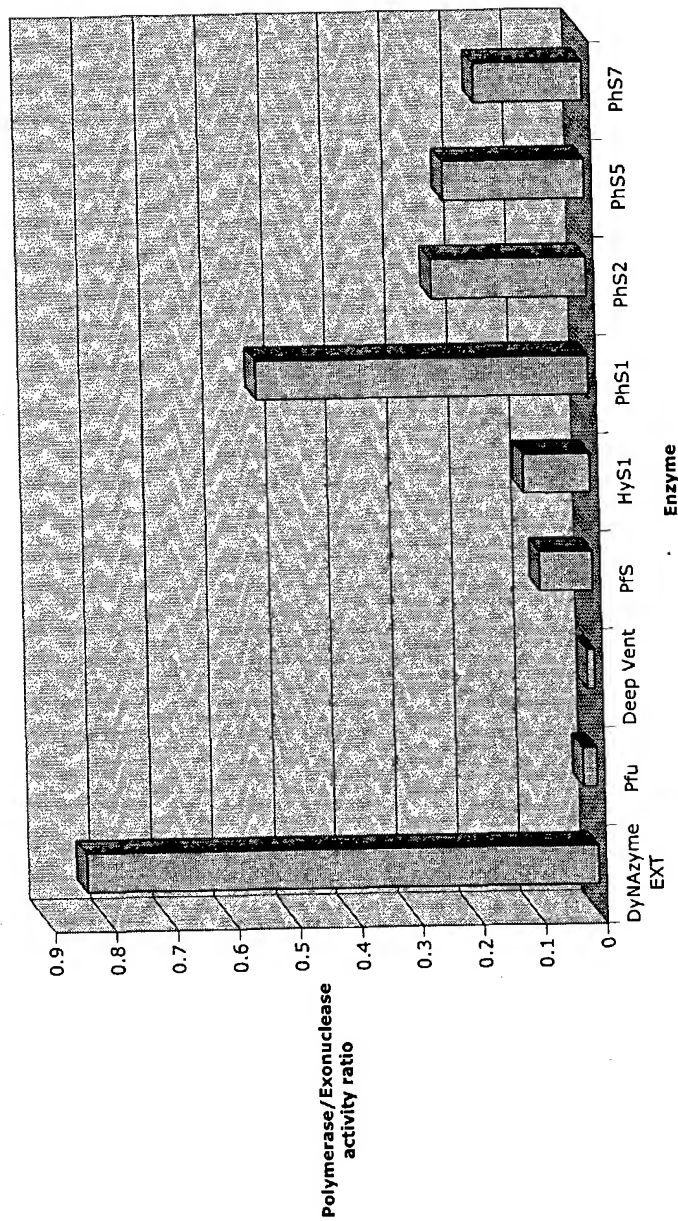


FIGURE 4

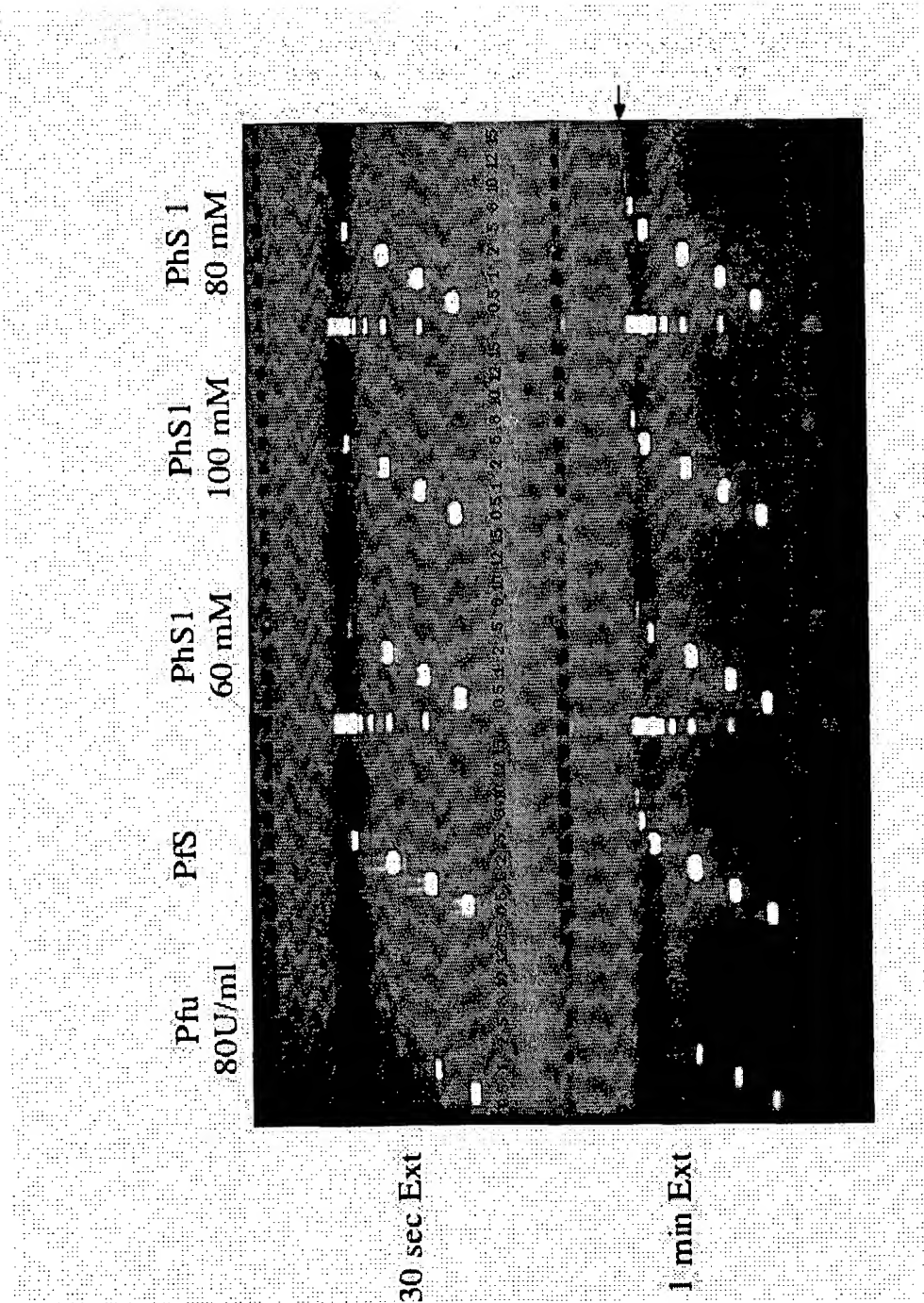


Figure 5.

Pfu	MILDVDYITTEEGKPVIRLFKKENGKFKLEHDTIRFPYIYALLRDDSQTSEVKKITGERHGO
DeepVent	MILDADYITTEEGKPIIRLFKKENGKFKVEYDRNFRPYIYALLKDDSQIDEVKKITGERHGO
Hybrid_design	MILDADYITTEEGKPIIRLFKKENGKFKVEYDRNFRPYIYALLKDDSQIDEVKKITGERHGO
HyS1	MILDADYITTEEGKPVIRLFKKENGKFKLEHDTIRFPYIYALLRDDSQTSEVKKITGERHGO
Hyb2	MILDADYITTEEGKPVIRLFKKENGKFKVEYDRNFRPYIYALLKDDSQIDEVKKITGERHGO
Hyb3	MILDADYITTEEGKPVIRLFKKENGKFKVEYDRNFRPYIYALLKDDSQIDEVKKITGERHGO
HyS4	MILDADYITTEEGKPVIRLFKKENGKFKLEHDTIRFPYIYALLRDDSQTSEVKKITGERHGO
PhS1	MILDADYITTEEGKPVIRLFKKENGKFKLEHDTIRFPYIYALLKDDSQIDEVKKITGERHGO
PhS2	MILDVDYITTEEGKPVIRLFKKENGKFKVEYDRNFRPYIYALLKDDSQIDEVKKITGERHGO
PhS3	MILDADYITTEEGKPIIRLFKKENGKFKVEYDRNFRPYIYALLKDDSQIDEVKKITGERHGO
PhS4	MILDADYITTEEGKPVIRLFKKENGKFKVEYDRNFRPYIYALLRDDSQTSEVKKITGERHGO
PhS5	MILDADYITTEEGKPIIRLFKKENGKFKVEYDRNFRPYIYALLRDDSQTSEVKKITGERHGO
PhS6	MILDADYITTEEGKPIIRLFKKENGKFKVEYDRNFRPYIYALLRDDSQTSEVKKITGERHGO
PhS7	MILDADYITTEEGKPIIRLFKKENGKFKVEYDRNFRPYIYALLRDDSQTSEVKKITGERHGO

Pfu	KIVRIYDVEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
DeepVent	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
Hybrid_design	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
HyS1	KIVRIYDVEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
Hyb2	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
Hyb3	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
HyS4	KIVRIYDVEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS1	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS2	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS3	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS4	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS5	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS6	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY
PhS7	KIVRIYDAEKVEKKFLGRPIITVWRLYLFHPODVPITREKIREHSAVVDIFEYDIPFAKRY

Pfu	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
DeepVent	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
Hybrid_design	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
HyS1	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
Hyb2	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
Hyb3	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
HyS4	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS1	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS2	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS3	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS4	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS5	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS6	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY
PhS7	LIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIMISYADENEAKVITWKKIDLPY

Pfu	VEVVSSEEREMIKRFLKVIIEKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
DeepVent	VEVVSSEEREMIKRFLKVIIEKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
Hybrid_design	VEVVSSEEREMIKRFLKVIIEKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
HyS1	VEVVSSEEREMIKRFLKVIIEKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
Hyb2	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
Hyb3	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
HyS4	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS1	VEVVSSEEREMIKRFLKVIIEKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS2	VEVVSSEEREMIKRFLKVIIEKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS3	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS4	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS5	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS6	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK
PhS7	VEVVSSEEREMIKRFLRVIREKDDPDVITVYNGDSFDLPYLAKRAEKLGIKLPGRDGESEPK

Pfu	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
DeepVent	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
Hybrid_design	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
HyS1	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
Hyb2	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
Hyb3	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
HyS4	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS1	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS2	MQRIGDMTAVEVKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS3	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS4	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS5	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS6	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE
PhS7	MQRIGDMTAVEIKGRIFHFDLYHVIRRTINLPTTYTLEAVYEAIFGKPKKVVYADEIAEAWAE



Pfu  
 DeepVent  
 Hybrid\_design  
 Hyb1  
 Hyb2  
 Hyb3  
 Hyb4  
 PhS1  
 PhS2  
 PhS3  
 PhS4  
 PhS5  
 PhS6  
 PhS7

610 620 630 640 650 660  
 ECGKIVITRGLEIVRRDWSFI AKETQAR VLEITLLKHGDNVEEAVRIVKEVITQKLANVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETQAK VLEAILLKHGDNVEEAVKIVKEVTEKLSKVEIPPEK  
 ECKKXITRGLEIVRRDWSFI AKETOAX VLEXILLKHGXVVEEAVXIVKEXXKXXVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEAILLKHGDNVEEAVKIVKEVTEKLAKEVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEAILLKHGDNVEEAVKIVKEVTEKLAKEVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEAILLKHGDNVEEAVKIVKEVTEKLAKEVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEAILLKHGDNVEEAVKIVKEVTEKLAKEVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEAILLKHGDNVEEAVKIVKEVTEKLAKEVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEAILLKHGDNVEEAVRIVKEVTEKLSKVEIPPEK  
 ECKKITTGELLEIVRRDWSFI AKETOAK VLEITLLKHGDNVEEAVRIVKEVTEKLAKEVEIPPEK

Sequence logo for the 10th position of the 100-residue protein. The y-axis lists protein variants: Pfu, DeepVent, HybVent, Hybrid\_design, HyS1, Hyb2, Hyb3, HyS4, PhS1, PhS2, PhS3, PhS4, PhS5, PhS6, and PhS7. The x-axis shows positions 85, 86, 87, 88, 89, and 90. The logo shows that for most variants, the 10th position is highly conserved as Lysine (K). The 'Hybrid\_design' variant shows a different pattern, with a strong preference for Lysine (K) at positions 85-87 and a mix of Lysine (K) and Asparagine (N) at positions 88-90.

Figure 6

